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MICROSCOPY.<sup>1</sup>

THE ALIMENTARY CANAL OF THE CRUSTACEA.—1. *Hardening*.—For the river cray fish, Frenzel<sup>2</sup> recommends Kleinenberg's picrosulphuric acid diluted with only two times its volume of water. The preparation is left fifteen minutes in the fluid, then treated with the usual grades of alcohol. Osmic acid and the various chromic solutions proved worthless. Perenyi's fluid caused a slight swelling, but was of some service in the study of the liver and the nuclei of the middle gut. Corrosive sublimate (saturated aqueous solution) proved an excellent means of isolating the epithelium of the middle gut in the lobster. In preparations hardened in this fluid the epithelium becomes loosened from the wall of the canal, so that it can be stripped off in sheets and prepared for surface examination.

2. *Imbedding*.—Paraffine is preferred to celloidin. Precaution should always be taken to prevent the formation of large crystals, which not only render the paraffine brittle, but also injure the finer structure of the preparation, by immersing it in cold water and cutting soon afterward. If the paraffine block is allowed to stand for weeks crystallization sets in.

3. *Staining*.—The sections are fixed on the slide with chrome mucilage, then stained with alum carmine, alcohol carmine (Grenacher), aqueous hæmatoxylin (Böhmer) and safranin. For the epithelium of the middle gut a double stain with acid carmine and hæmatoxylin offers some advantages.

FRENZEL'S CHROME MUCILAGE AS A FIXATIVE.<sup>3</sup>—Make a thin solution of gum arabic in water and add to this an aqueous solution of chrome alum. An excess of the latter does no harm. A little glycerine is added to the mixture to prevent it from drying too rapidly when painted on the slide.

After painting the slide with a small brush the sections are placed in order and the slide left for a few minutes (not over fifteen minutes) in the oven of a water-bath kept at 30–45° C. The gum is thus rendered insoluble. The paraffine is next removed in the ordinary way and the sections stained according to desire. Fuchsin and safranin are the only aniline dyes which cannot be used, as they stain the film of gum deeply and thus injure the preparation.

THE RETRACTILE TENTACLES OF THE PULMONATA.—The retraction of the eye-bearing tentacles of a land snail in the process of hardening may be prevented in the manner described by Flemming.<sup>4</sup> These organs, as is well known, are hollow cylinders,

<sup>1</sup> Edited by Dr. C. O. WHITMAN, Mus. Comparative Zoology, Cambridge, Mass.

<sup>2</sup> Arch. f. mik. Anat., xxv, p. 141–143, July, 1885.

<sup>3</sup> Arch. f. mik. Anat., xxv, p. 52, April, 1885.

<sup>4</sup> Arch. f. mik. Anat., 1870, vi, p. 440; Zeitschr. f. wiss. Zool., 1872, xxii, p. 356.

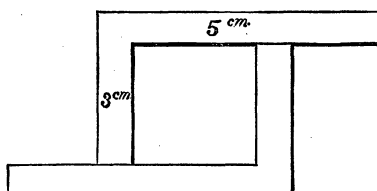
which can be rolled in like the finger of a glove by the action of a retractor muscle. The eversion is effected by forcing blood from the body-cavity into the lumen of the tentacle. If, when the tentacle is fully extended, a thread be quickly tied around its base so as to prevent the escape of the blood, the infolding process cannot take place, and the hardening fluid may then be safely applied. Flemming recommends treatment with bichromate of potassium (4 p. c.). This method enables one to obtain sections of the eye in the extended condition of the tentacle.

**IMBEDDING IN PARAFFINE.**—*Clarifying media.*—Cedar-wood oil is recommended by Lee<sup>1</sup> as a clarifying medium on various grounds, the more important of which are the following:

1. It clarifies as rapidly as clove oil.
2. It does not render the object brittle or excessively hard.
3. It is a much better solvent of paraffine than clove oil; and hence only a short paraffine bath is required.
4. The penetration of the paraffine is not only more rapid, but also more thorough.

Holl<sup>2</sup> recommends toluol, which he finds can be used to greater advantage than chloroform, especially with large objects. The largest objects should be left one day in toluol, and one day in the paraffine bath; small objects require correspondingly shorter times.

*Imbedding Box.*—A convenient box, introduced by Dimmock, may be made of two pieces of type metal (or better of brass). As will be seen from the accompanying diagram, each piece of



metal has the form of a carpenter's square. A convenient size will be found in pieces measuring 5<sup>cm</sup> (long arm) by 3<sup>cm</sup> (short arm) and 7<sup>mm</sup> high. With such pieces a box may be constructed at any moment by simply placing them together on a plate of glass which has previously been wet with glycerine, and gently warmed. The area of the box will evidently vary according to the position given to the pieces, but the height can be varied only by using different sets of pieces.

It is well to imbed in a thin layer of paraffine, so that the object, after cooling, may be cut out in small cubical or pyramidal

<sup>1</sup> Zool. Anz., No. 205, p. 563, Oct., 1885.

<sup>2</sup> Zool. Anz., No. 192, p. 223, April, 1885.

blocks, which may be easily fixed, for cutting, to a larger block of hard paraffine, or better, to a block of wood saturated with paraffine.

ORIENTATION WITH SMALL OBJECTS.—Orientation becomes difficult only with objects so small that their position can be controlled only by the aid of a microscope. Spherical objects, less than one millimeter in diameter, *e. g.*, many ova and embryos, are the most difficult to manage. Such objects may usually be successfully oriented in the following manner:

1. Prepare the box; for this it will be necessary to use the two triangular pieces of metal, a rectangular glass plate (2 in. x 2½ in.). The plate should be cleaned and then smeared with glycerine, and the pieces of metal so adjusted, that the arms are parallel with the edges of the plate.

2. Having warmed the box over a spirit lamp, lift the object from the basin of paraffine by the aid of a *small, flat, thin* spatula (first starting it from the bottom by shaking the paraffine a little), and allow it to *flow* with the paraffine carried on the spatula into the box.

3. Then fill the box (5–6<sup>mm</sup> deep) with the melted paraffine, and warm it a little over a spirit lamp, just enough to keep *all* of the paraffine in a liquid condition for a few moments. Now place the box on the warm table of a dissecting microscope, and by the aid of a hot needle proceed to place the object in the desired position. As the object is illuminated from below, it can be easily seen, turned over, and moved about at pleasure. If the paraffine freezes before orientation is effected, it should be melted again as before, and the needle must be kept hot by repeatedly holding it in the flame of the lamp.

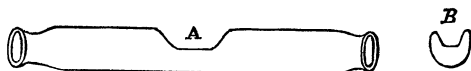
The difficulty of finding very small objects in a basin of paraffine will be very much lessened by keeping the paraffine free from dust, and the bottom of the basin (tin) scoured bright. A piece of emery cloth serves for polishing.

The necessity of re-warming the box of paraffine, which often arises in the above method, may be removed by using a hot bath on the table of the microscope. This bath should be a box of convenient size (not over 2<sup>cm</sup> high), with top and bottom of glass, with an opening at one end for filling with hot water, and another at the opposite end provided with a rubber tube and clamp, for drawing off the water as soon as the object has been arranged.

PREVENTION OF BUBBLES.—After the imbedding process has been carried thus far, there is still another danger to be carefully guarded against. If the box is left to cool slowly in the air, bubbles are very likely to appear in the paraffine, which will prove a serious obstacle in cutting. Profiting by Caldwell's suggestion, to cool the box in water, one may avoid all such inconveniences. As soon as the paraffine cools around the object, so

that its position is secured, the box should be held in a vessel of cold water, first at the surface (until the paraffine has set), then fully submerged. In this way the paraffine is quickly cooled sufficiently for removal from the box, which may then be used for imbedding a second object. A dozen objects may be thus imbedded in a very short time. If the box is plunged below the surface of the water, before the paraffine has become rigid, holes will arise in the mass and fill with water.

**BOX AND WARM BATH COMBINED.**—Selenka<sup>1</sup> has recently described a simple apparatus for imbedding small objects in a definite position. It is made by taking a thin glass tube, and heating one side at the middle until it becomes soft enough to bend easily. Then the mouth is applied to one end while stopping the other with the finger, and the air sucked out, causing the softened portion to bend inward and thus forming a shallow trough, A. A section of the trough is shown in B. In order to make the bottom of the trough flat, it is only necessary to place in the tube, before



Selenka's Imbedding Box (reduced  $\frac{1}{2}$ ).

heating, a strip of glass, against which the infolding portion of the tube will flatten.

One end of the cylinder is joined by rubber tubing with a T-shaped tube, one arm of which connects with a somewhat elevated liter glass of cold water, and the other arm with the hot water of the water-bath. The connection with the water reservoirs is through rubber tubing, which can be closed by pinch-corks. The other end of the glass cylinder is also provided with a rubber tube through which the water flows into a vessel standing on the floor.

The cylinder is fastened (by string or clamp) to the table of a dissecting microscope, with the trough at the centre. The hot water is then turned on and allowed to flow slowly. The trough is next filled with melted paraffine by the aid of a hot pipette, and the object dropped in and oriented with a needle. As soon as the object is placed in the desired position, the cold water is turned on, which causes an immediate stiffening of the paraffine. The hot water is shut off, and after a few moments, the paraffine with the imbedded object can be removed from the trough. The trough should be washed with absolute alcohol before imbedding.

<sup>1</sup> Zool. Anz., No. 199, p. 419, July, 1885.